

Research paper

Sociodemographic and clinical correlates of HSV2 infection among antenatal clinic attendees in Benin.

ABSTRACT

BACKGROUND: Recognition of the clinical correlates of HSV-2 infection by pregnant women will prompt health-seeking activity which will improve case detection and also enable informed interventions aimed at preventing some of the various complications that are associated with the infection among pregnant women. There's yet no documented data on the patient-recognizable clinical correlates and the sociodemographic factors associated with the occurrence of genital herpes among pregnant women in Nigeria. These data will guide the design of public health interventions and will provide evidence-base for the formulation of more effective guidelines for case management.

OBJECTIVES: To identify sociodemographic and clinical correlates of HSV-2 infection among pregnant women attending ante-natal clinics in Benin, Nigeria.

STUDY LOCATION, DESIGN AND DURATION: All the participants were recruited from the two major hospitals in Benin: University of Benin Teaching Hospital and Central Hospital, Benin. The cross-sectional study took place between November 2011 and June 2012.

METHODOLOGY: Six hundred and seventy four (674) consenting ante-natal clinic attendees were consecutively and prospectively included. Their sociodemographic and obstetric data were obtained by the use of structured questionnaires and hospital case records. Each participant's serum was analyzed for HSV-2 IgG antibodies by gG-based type-specific ELISA. Counselling and testing for HIV was also done. Data analysis was done using SPSS version 16.

RESULTS: The average age of the 674 participants was 30.6 ± 5.2 years; and most of them were married and had complete secondary education. Seroprevalence of HSV-2 was 47.3%. Factors that were significantly associated with HSV-2 infection included age, level of education and parity. HIV infection and positive history of sexually transmitted infections were significantly associated with HSV-2 infection. The HSV-2-infected were significantly more likely to recall episodes of genital rashes, vaginal discharge and urethral discharge.

CONCLUSION: Risk factors for HSV-2 infection among pregnant women could include increasing age, higher parity, education below secondary level, HIV-positive status, and positive history of sexually transmitted infections. History of genital rashes, vaginal discharge and urethral discharge syndromes were associated with HSV-2 infection.

KEY WORDS: Seroprevalence, Human herpes virus 2, sociodemographic, syndromes, sexually transmitted infections (STI's), risk factors

1.0 INTRODUCTION

Human Herpesvirus 2 (HSV-2) has been described as a silent pandemic with different countries being at different stages of the epidemic.[1,2] Humans are the only natural host .[3] Although most cases are asymptomatic, transmission through viral shedding still goes on. [4,5]

HSV-2 infection has been reported to complicate HIV control and obstetric outcomes. [6,7] Since HSV-2 infection is known to be so asymptomatic in most cases[8], and obstetric complications tend to be the sole pointer to the prior existence of the infection[8,9,10], improved case detection will contribute substantially to its control. In such a resource-poor settings where routine screening of all pregnant women is not feasible, the ability of the pregnant women to identify symptoms of the infection can be a useful starting point in presumptive detection of cases (primary and recurrent). There is need to assess the symptoms, as recognised by the patient, that are associated with HSV-2 infection in this environment. Such information is potentially vital in resource-poor settings as it will help in defining cases clinically; and in the subsequent implementation of case-specific interventions. It could also become an essential tool for enlightenment campaigns; and could further form a basis for selection of cases for targeted screening.

Since HSV-2 infection is life-long and has no known cure, primary prevention is the mainstay of its control.[11] Epidemiological data is required for an effective primary prevention; and there's dearth of data on the epidemiology of this infection among pregnant women in Nigeria, although there are some documentations on the infection among commercial sex workers and among sexually transmitted infection clinic attendees[12,13].

This study is aimed at determining the sociodemographic and clinical correlates of Human Herpesvirus 2 infection among pregnant women in Benin, Nigeria.

2.0 METHODOLOGY

2.1 Study Location, Design and Duration: The participants were ante-natal clinic attendees of the two major tertiary hospitals in Benin metropolis: University of Benin teaching Hospital(UBTH) and Central Hospital, Benin-City(CHB). UBTH serves as a major referral centre for Edo State, Ondo state and Delta States, while CHB is transiting to a teaching hospital and subserves secondary and tertiary health care delivery for Benin-Capital-City and other parts of Edo State. The cross-sectional study took place between November 2011 and June 2012.

2.2 Data Collection: The 674 consenting participants were consecutively and prospectively recruited.

Structured questionnaire was used to collect data on specific participants' sociodemographic, and clinical profiles. Obstetric data was obtained from hospital case records. Blood samples were obtained from the participants and the serum was separated and used for glycoprotein G-based type-specific assay for HSV-2 IgG antibodies.

2.2.1 Laboratory Procedures: Blood samples were collected in 5 ml plain vacutainer tubes and allowed to clot and sera separated by centrifugation at room temperature. Storage was in cryovials at -20°C.

2.2.1.1 HSV-2 assay procedure: HSV-2 IgG assay utilized Enzyme Linked Immunosorbent Assay (ELISA) kit by Dia. Pro. Diagnostic Bioprobes Milano – Italy. This is a glycoprotein G-based enzyme-linked immunosorbent assay (ELISA) technique. All specimens and kit reagents were brought to room temperature and gently mixed before the assay. Procedures were performed in accordance with instructions in the kit manual. Each batch of tests ran with both positive and negative controls and results were qualitative.

2.2.1.2 HIV immunoassay procedure: The HIV statuses of the respondents were previously determined using Determine ® HIV 1/2 by Inverness Medical Innovations South Africa; and HIV 1 & 2 STAT PAK Assay kit by CHEMBIO Diagnostic system, INC, New York, USA in accordance with Nigerian Federal Ministry of Health guidelines[14].

2.3 Data Analysis

84 Data collected was analyzed using the SPSS version 16 computer software. Fishers exact test and Chi²
85 was used to test associations. Statistical significance was ascribed based on P values < 0.05.

86 **3.0 RESULTS AND DISCUSSION**

87 **3.1 Sociodemographic and obstetric characteristics of participants:** The age range of the
88 674 participants was 18 years to 44 years. Their mean age was 30.6 ± 5.2 years.
89 Majority (96.6%) of the participants were in the 20-40 age range; and most (85.2%) were married. They
90 were either Christians or Muslims, in the ratio was 4.7 : 1 respectively.

91 Most of the members of the baseline study population had good education. Only 6.5% did not achieve
92 complete secondary education. (Table 1).

93 The occupations of 61.7% of the participants were trading/private business operations, teaching, and
94 clerical work. next in frequency. 24.2% of them were either students, housewives or unemployed. The
95 least represented occupation was farming, 0.3%(Table 2)

96 Most (43.5%) of the participants were nulliparae. Twenty-eight percent (28.2%) of them were
97 primiparae; while a total of 27.6% of them were multi-parae. Only two (0.7%) of the participants
98 were grand multiparae. (Table 3)

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TABLE 1: GENERAL CHARACTERISTICS OF PARTICIPANTS

CHARACTERISTICS	FREQUENCY	PERCENT (%)
AGE GROUP (YEARS)		
15-20	7	1.0
21-25	98	14.5
26-30	242	35.9
31-35	225	33.4
36-40	86	12.8
41-45	16	2.4
RELIGIONS		
CHRISTIANITY	556	82.5
ISLAM	118	17.5
MARITAL STATUSES		
MARRIED	574	85.2
SINGLE	53	7.8
DIVORCED	22	3.3
WIDOWED	25	3.7
LEVELS OF EDUCATION		
GRADUATE AND ABOVE	210	31.2
POST-SECONDARY	292	43.3
SECONDARY COMPLETED	128	19.0
SECONDARY UNCOMPLETED	14	2.1
PRIMARY COMPLETED	27	4.0
PRIMARY UNCOMPLETED	3	0.4

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TABLE 2: OCCUPATION OF PARTICIPANTS

OCCUPATION	FREQUENCY	PERCENT
HOUSEWIVES	51	7.6
UNEMPLOYED	41	6.0
STUDENTS	65	9.6
FARMERS	2	0.3
TRADERS/PRIVATE BUSINESS		
OPERATIVES	215	31.9
SECRETARIES/CLERKS/TYPISTS	100	14.8
FASHION DESIGNERS/HAIR		
STYLISTS/TAILORS	60	8.9
ACCOUNTANTS/FINANCE		
INSTITUTION PROFESSIONALS	20	3.0
TEACHERS	102	15.1
NURSES	7	1.0
DOCTORS	8	1.2
PHARMACISTS	3	0.4
TOTAL	674	100

TABLE 3: OBSTETRIC CHARACTERISTICS OF ALL PARTICIPANTS

CHARACTERISTICS	FREQUENCY	PERCENT
GESTATIONAL AGES ON RECRUITMENT		
1 ST TRIMESTER	99	14.7
2 ND TRIMESTER	268	39.8
3 RD TRIMESTER	307	45.5
PARITY		
NULLIPARA	293	43.5
PRIMIPARA	190	28.2
PARA-2	123	18.2
PARA-3	43	6.4
PARA-4	20	3.0
PARA-5 OR MORE	5	0.7

3.2 HSV-2 and HIV Statuses of Participants

HSV-2 antibodies was present in 46.3% of the participants; while the prevalence of HIV antibodies was 12.5%. (Table 4)

TABLE 4: PRESENCE OF HSV-2 AND HIV ANTIBODIES AMONG ALL THE PARTICIPANTS

ANTIBODIES	FREQUENCY(N=674)	PERCENT
HSV-2 ANTIBODIES		
Positive	312	46.3
Negative	362	56.7
HIV ANTIBODIES		
Positive	84	12.5
Negative	590	87.5

TABLE 5: PARTICIPANTS' CHARACTERISTICS AND THEIR ASSOCIATION WITH HSV-2 INFECTION

CHARACTERISTICS	HSV-2 ANTIBODIES PRESENT (%)	TOTAL NO. TESTED	P-VALUE
AGE			
15-20	2(28.6)	7	P = .001
21-25	38(38.8)	98	
26-30	96(39.7)	242	
31-35	109(48.4)	225	
36-40	59(68.6)	86	
41-45	8(50)	16	
MARITAL STATUS			
Married	245(43.2)	574	P = .003
Single	33 (71.4)	53	
Divorced	17 (70.8)	22	
Widowed	17 (68.8)	25	
LEVEL OF EDUCATION			
University graduate and above	93(44.3)	210	P = .001
Post-secondary	129(44.2)	292	
Secondary completed	60(46.9)	128	
Secondary uncompleted	14(100)	14	
Primary completed	16(59.3)	27	
Primary uncompleted	0(0)	3	
RELIGION			
Christianity	250(45.0)	556	P = .16
Islam	62(52.5)	118	
PARITY			
Nullipara	72(40.4)	293	P = .004
Primipara	57(49.1)	190	
Para-2	37(50)	123	
Para-3	18(66.7)	43	
Para-4	10(83.3)	20	
Para-5 or more	0(0)	5	
HIV-STATUS			
POSITIVE	36(69.2)	84	P = .001
NEGATIVE	158(44.1)	590	

118 There was a general increase in prevalence of HSV-2 infection with increasing age. Age was significantly
119 associated with HSV-2 infection. (p=0.001).

120 The infection occurred significantly more among all categories of unmarried participants, than among the
121 married. (p = 0.003)

122 The prevalence was highest among participants who did not attain complete secondary education. Level
123 of education was significantly associated with the occurrence of HSV-2 infection.(p = 0.001)

124 There was no significant association between occurrence of the occurrence of the infection and religion.(p
125 = 0.16)

126 The prevalence of the infection was generally higher among multiparous participants relative to the
127 nulliparous ones; and the relationship between parity and HSV-2 infection was statistically significant.(p =
128 0.004)(Table 4)

129 The prevalence of HSV-2 infection was greatest among the unemployed and the pharmacists; and least
130 among the farmers. (Table 6)

131 **TABLE 6: OCCUPATIONAL GROUPS AND PREVALENCE OF HSV-2 ANTIBODIES**

OCCUPATION	NUMBER TESTED (%)	HSV-2 ANTIBODY POSITIVE(%)
HOUSEWIVES	34	19 (55.9)
UNEMPLOYED	29	24(82.8)
STUDENTS	65	23(35.4)
FARMERS	2	0(0)
TRADERS/PRIVATE BUSINESS OPERATIVES	215	97(45.1)
SECRETARIES/CLERKS/TYPISTS	100	50(50.0)
FASHION DESIGNERS/HAIR STYLISTS/TAILORS	60	31(51.7)
ACCOUNTANTS/FINANCE INSTITUTION PROFESSIONALS	20	10(50.0)
NURSES	7	3(42.9)
DOCTORS	8	3(37.5)
PHARMACISTS	3	3(100)
TEACHERS	102	49(48.0)
TOTAL	674	312

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HSV-2 infection was significantly more prevalent among the HIV-infected than among the HIV-uninfected. (p=0.001). (Table 5)

Majority (73.4%) of the participants had a positive history of sexually transmitted infections and the prevalence of HSV-2 infection was significantly higher in this group than in the group without history of sexually transmitted infections (p = .001).(Table 7)

TABLE 7: HISTORY OF SEXUALLY TRANSMITTED INFECTIONS (STI's) AND HSV-2 INFECTION

		HSV-2 ANTIBODIES		TOTAL
		POSITIVE	NEGATIVE	
HISTORY OF STI'S	POSITIVE	277(56.0)	218 (44.0)	495(73.4)
	NEGATIVE	35(19.6)	144 (80.4)	179 (26.6)
	TOTAL	312	362	674

Chi² = 70.0; df = 1; p = .001

Only small proportions of the participants (11.2%) could recall episodes of urethral discharge. HSV-2 antibody was significantly more prevalent among those with positive history of urethral discharge syndrome than those with negative history (p = 0.012).

Forty percent (40%) of the participants could recall episodes of abnormal vaginal discharge. The prevalence of HSV-2 antibody was higher among those that had positive history of abnormal vaginal discharge than those with negative history (p = 0.001).

Only 9% of the participants could recall having had painful genital ulcers. There was no significant association between the positive history of genital ulcers and occurrence of HSV-2 infection (p = 0.490)

Majority (56.8%) of the participants could recall having had rashes in the genital region; the prevalence of HSV-2 infection was more in this subgroup; and the difference was statistically significant (p = 0.001).(Table 8)

TABLE 8: HISTORY OF SEXUALLY TRANSMITTED INFECTION (STI) SYNDROMES AND HSV-2 INFECTION

HISTORY OF STI SYNDROMES	HSV-2 ANTIBODIES POSITIVE (%)	TOTAL NUMBER TESTED (%)	P-VALUE
URETHRAL DISCHARGE			
POSITIVE	47(63.5)	74(11.2)	P = .012
NEGATIVE	265(44.2)	600(88.8)	
ABNORMAL VAGINAL DISCHARGE			
POSITIVE	186(70.5)	264(40.0)	P = .001
NEGATIVE	126(30.7)	410(60.0)	
PAINFUL GENITAL ULCERS			
POSITIVE	24(38.7)	62(9.2)	P = .23
NEGATIVE	288(47.1)	612(90.8)	
GENITAL RASHES			
POSITIVE	223(58.4)	382(56.8)	P = .001
NEGATIVE	89 (30.5)	292(43.2)	

4.0 DISCUSSION

The 46.3% HSV-2 seroprevalence found in the study population is high; and is higher than the 20.7%, 33.6% and 26% found among pregnant populations in two respective sub-Saharan African countries, Tanzania and Senegal [15-17]. This finding is also a lot higher than 7.5%, 11.5%, 14.5% and 22.0% found among pregnant populations in India, Australia, Mexico and USA[18-21].

Certain socio-demographic and behavioral factors were assessed for their relevance in determining the prevalence of this infection in this environment. Age was one of the socio-demographic variables found to be significantly associated with increased prevalence of HSV-2 infection. This association is attributable to the fact that HSV-2 seroprevalence simply accumulates in a community with time (as long as transmission continues), since the infection is lifelong.[11] Similar observations have been made in several previous studies among persons of reproductive age.[19,22] This result suggests that control measures for HSV-2 infection should commence at early years.

Increased prevalence of HSV-2 infection was also significantly associated with education below secondary school level and unmarried statuses – findings that were corroborated by reports from Brazil and Croatia.[23,24] The finding on the effect of education could be attributable to the well-known effect of ignorance in determining the distribution of disease; while the finding on effect of marital status could be due to the unfaithful sexual behaviours that often characterize unmarried relationships. This effect of marital status is corroborated by a study report from Croatia [24]. HSV-2 antibodies were most prevalent among the unemployed, possibly due to indiscrete sexual behavior, which could be encouraged by unemployment and the associated poverty. Similar finding of association with unemployment was also reported from Croatia [24]. In this study, multiparity was also found to be significantly associated with increased HSV-2 seroprevalence, as has also been reported in India and USA.[18,25] It, therefore, follows that age-above-30, education below secondary level, unemployed status, unmarried status, and multiparity ought to be taken into consideration while designing primary prevention strategies.

Furthermore, assessment of these factors in individual case management could help in raising the index of suspicion of HSV-2 infection and are potentially useful screening criteria, if validated. Such screening protocol could incorporate positive HIV status, positive history of sexually transmitted infections, positive history of abnormal vaginal discharge, and positive history of genital rashes, all of which were found to be significantly associated with HSV-2 infection in this study.

There is a need to define the clinical features that predict HSV-2 infection in order improve case recognition and guide public enlightenment campaigns[1]. We found that positive history of genital ulcers was not highly prevalent in this population and there was no significant association of genital ulcers with HSV-2 antibodies, in this study. This result agrees with a study report from India in which only one of the HSV-2 infected participants had history of genital ulcer disease [26]. Moreover, LGV, and not genital herpes, was the major cause of GUD in a group of Nigerian females [27]. It could be that the high prevalence of HSV-1 in Nigeria protects the population from severe mucocutaneous pathologies [1].

Close to half of the participants recalled having had rashes in the genital region (genital rashes) and this feature was found to be significantly associated with HSV-2 infection. The effect of 'genital rashes' was assessed because previous reports had shown painful genital ulcer as infrequent among the HSV-2-infected[26]. Moreover, 'genital rashes', though not a classical STI syndrome, is a clinically feature that could be easily understood by the participants. Case detection and recognition by the patients could improve if occurrence of genital rashes is promoted as an indication for health-seeking activity.

The typical sexually transmitted infection syndromes that were more significantly associated with HSV_2 infection were urethral discharge and vaginal discharge. In a previous study among pregnant women in India, vaginal discharge was the most frequent feature among the HSV-2-infected [28]; while previous history of urethritis of *Chlamydia spp* and *Trichomonas spp.* origin, were significantly associated with HSV-2 infection as reported in Australia and India[19,27]. Moreover, bacteria vaginosis has been shown to significantly increase the risk of acquisition of HSV-2 in a previous report [26-29]. There is need to

evaluate and validate the combination of vaginal discharge, urethral discharge, and genital rashes as a clinical screening tool.

The previously reported links between HIV infection and HSV-2 infection was further highlighted by this study[30]. Efforts to detect and control HSV-2 should be intensified as it translates to more effective HIV control [31].

CONCLUSIONS

The prevalence of HSV-2 infection among pregnant women in Benin is high. Prevention remains the potent means of controlling HSV-2 infection and the associated complications. Therefore, socioeconomic developmental challenges require addressing. Public health campaigns aimed at encouraging good health-seeking behavior on noticing genital rashes, urethral discharges, and vaginal discharges are recommended.

COMPETING INTERESTS

No competing interests exist.

CONSENT

Written informed consent was obtained from the participants for carrying out and publishing this research work.

ETHICAL APPROVAL

Approval was obtained from the ethical committee of University of Benin Teaching Hospital, Benin for carrying out this study.

REFERENCES

1. Looker KJ, Garnett GP, Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bull World Health Organ* 2008; 86(10): 737-816.
2. Oni AA, Adu FD, Ekweozor CC, Bakare RA. Genital herpes simplex virus infection in females in Ibadan Nigeria. *West Afr J Med*. 1996;15(2):107-10.
3. Dordevic H. Serological response to herpes simplex virus type 1 and 2 infection among women of reproductive age. *Med Pregl*. 2006[cited 2013 01 12] ; 59(11-12):591-7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17633904>
4. Corey L, Handsfield HH. Genital Herpes and public health: addressing a global problem. *JAMA* 2000; 283: 791-794.
5. Kolawole O M, Adu. FD, Agbede OO, Oni AA, Bakare RA. Epidemiological Patterns of Human Immunodeficiency Virus and Herpes Simplex Virus Co-Infection in Ibadan, Nigeria. *Afr. J. Biomed Res*. 2008; 11: 23 -26.
6. Torok E, Moran E, Cooke F. Congenital infections. In: *Oxford Handbook of Infectious Diseases and Microbiology*. 1st edition. New York: Oxford University Press; 2009.pp.824 – 826.
7. Centers for disease Control and Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Division of STD Prevention. Sexually transmitted diseases: Genital Herpes- CDC fact sheet. 2013. URL:<http://www.cdc.gov/std/Herpes/STDFact-Herpes.htm>

8. Duran N, Yarkin F, Evruke C, Koksall F. Asymptomatic herpes simplex virus type 2 (HSV-2) infection among pregnant women in Turkey. *Indian J Med Res.* 2004;**120**(2):106-10.
9. Sandhaus S. Genital herpes in pregnant and non-pregnant women. *Nurse Pract.* 2001 Apr;**26**(4):15-6, 21-22, 25-27. Available from: URL:http://findarticles.com/p/articles/mi_qa3958/is_200104/ai_n8946495/
10. Jerome KR, Morrow RA. Herpes simplex viruses and Herpes B virus. In: Murray PR, editor. *Manual of Clinical microbiology*, volumell. 9th edition. Washington: ASM; 2007. P. 1523-1536.
11. Center for Disease Control and Prevention. Seroprevalence of Herpes Simplex Virus Type 2 Among Persons Aged 14–49 Years --- United States, 2005—2008. *Morbidity and Mortality Weekly Report (MMWR)*, **2010**[cited 2013 01 24 ; **59**(15);456-459. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5915a3.htm>
12. Dada AJ, Ajayi AO, Diamondstone L. A sero-survey of *Haemophilis ducreyi*, Syphilis, and Herpes simplex virus type-2 and their association with HIV among sex workers in Lagos, Nigeria. *Sex Transm Dis* 1998[cited 2011 04 01]; **25**: 237–42. Available from: URL: <http://www.ncbi.nlm.nih.gov/pubmed/9587174>
13. Agabi YA, Banwat EB, Mawak JD, Lar PM, Dashe N, Dashen MM, *et al.* Seroprevalence of herpes simplex virus type-2 among patients attending the Sexually Transmitted Infections Clinic in Jos, Nigeria *J Infect Dev Ctries* 2010; **4**(9):572-575. <http://jids.org/index.php/journal/article/viewDownloadInterstitial/21045370/439>
14. Federal Ministry of Health (FMOH) . National Guidelines for HIV and AIDS Treatment And Care in Adolescents and adults. Federal Ministry Of Health Abuja – Nigeria, 2010.
15. Yahya-Malima KI, Evien-Olsen B, Matee MI, Fykenes K and Haans L. HIV-1, HSV-2 and syphilis among pregnant women in a rural area of Tanzania: prevalence and risk factors. *BMC infect Dis* 2008[cited 2011 12 16]; **8**:75. Doi: 10.1186/1471- 2334-8-75.
16. Msuya SE, Uriyo J, Hussain A, Mbizvo EM, Jeansson S, Sam NE, *et al.* Prevalence of sexually transmitted infections among pregnant women with known HIV status in northern Tanzania. *Reproductive Health* 2009[2013 06 21]; **6**:4 doi:10.1186/1742-4755-6-4.
17. Diawara S, Kane CT, Legoff J, Gaye AG, Mboup S. Low seroprevalence of Herpes simplex virus type 2 among pregnant women in Senegal. *International J of STD and AIDS* 2008; **19**(3): 159-160. Available from: URL:<http://www.urbanreproductivehealth.org/publications/low-seroprevalence-herpes-simplex-virus-type-2-among-pregnant-women-senegal>.
18. Rathore S, Jamwal A, and Gupta V. Herpes simplex virus type 2: Seroprevalence in antenatal women. *Indian J Sex Transm Dis.* 2010 Jan-Jun; **31**(1): 11–15. doi: [10.4103/0253-7184.68994](https://doi.org/10.4103/0253-7184.68994)
19. Tideman RL, Taylor J, Marks C, Seifert C, Berry G, Trudinger B, *et al.* Sexual and demographic risk factors for herpes simplex type 1 and 2 in women attending an antenatal clinic. *Sex Transm Infect.* 2001 Dec;**77**(6):413-5. Available from:<http://www.ncbi.nlm.nih.gov/pubmed/11714937>
20. Herrera-Ortiz A, Conde-Glez CJ, Vergara-Ortega DN, García-Cisneros S, Olamendi-Portugal L, and Sánchez-Alemán MA, *et al.* Avidity of Antibodies against HSV-2 and Risk to Neonatal Transmission among Mexican Pregnant Women. *Infectious Diseases in Obstetrics and Gynecology* 2013[2013 08 13]; 2013: 1-6. Available from: <http://dx.doi.org/10.1155/2013/140142>
21. Xu F, Markowitz LE, Gottlieb SL, Berman SM. Seroprevalence of herpes simplex virus types 1 and 2 in pregnant women in the United States. *Am J Obstet Gynecol.* 2007 Jan;**196**(1):43.e1-6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17240228>
22. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J Infect Dis* 2002[cited 2011 02 14]; **186**: S3-28 doi: [10.1086/343739](https://doi.org/10.1086/343739).
23. Smith JS, Herrero R, Muñoz N, Eluf-Neto J, Ngelangel C, Bosch FX, Ashley RL. Prevalence and risk factors for herpes simplex virus type 2 infection among middle-age women in Brazil and the Philippines. *Sex Transm Dis.* 2001;**28**(4):187-94. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11318248>
24. Miskulin M, Miskulin I, Milas J, Antolović-Pozgain A, Rudan S, Vuksić M. Prevalence and risk factors for herpes simplex virus type 2 infections in East Croatia. *Coll Antropol.* 2011 Mar;**35**(1):9-14. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21661348>
25. Buchacz K, McFarland W, Hernandez M, Klausner JD, Page-Shafer K, Padian N, *et al.* Prevalence and correlates of herpes simplex virus type 2 infection in a population-based survey of young women in low-income neighborhoods of Northern California. *The Young Women's*

- Survey Team. [Sex Transm Dis](#). 2000 Aug;27(7):393-400. Available from :
<http://www.ncbi.nlm.nih.gov/pubmed/10949430>
26. Madhivanan P, Krupp K, Chandrasekaran V, Karat C, Arun A, Klausner JD, et al. The Epidemiology of Herpes Simplex Virus Type-2 Infection Among Married Women in Mysore, India. Sexually Transmitted Diseases, November 2007, Vol. 34, No. 11, p.000–000. DOI: 10.1097/OLQ.0b013e3180ca965a
27. Fawole OI, Okesola AO, Fawole AO. Genital ulcers disease among sexually transmitted disease clinic attendees in Ibadan, Nigeria. *Afr J Med Med Sci*. 2000 Mar;29(1):17-22. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11379461>
28. Biswas D, Borkakoty B, Mahanta J, Walia K, Saikia L, Akoijam BS. Seroprevalence and risk factors of Herpes Simplex virus type-2 infection among pregnant women in North-East India. *BMC Infectious Diseases* 2011; **11**: 325.
29. Cherpes TL, Meyn LA, Krohn MA, Hillier SL. Risk Factors for Infection With Herpes Simplex Virus Type 2: Role of Smoking, Douching, Uncircumcised Males, and Vaginal Flora. *Sexually Transmitted Diseases* 2003; **30** (5):405-410. Available from: http://journals.lww.com/stdjournal/Abstract/2003/05000/Risk_Factors_for_Infection_With_Herpes_Simplex.6.aspx
30. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. *J Infect Dis* 2002;185:45-52.
31. Watson-Jones D, Weiss HA, Rusizoka M, Chagalucha J, Baisley K, Mugye K, et al. Effect of Herpes Simplex Suppression on Incidence of HIV among Women in Tanzania. *N Engl J Med* 2008 [cited 2013 06 24]; 358:1560-1571. DOI: 10.1056/NEJMoa0800260